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Diet composition exacerbates or attenuates soman toxicity in rats: Implied metabolic control of nerve agent toxicity

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ABSTRACT

To evaluate the role of diet composition on nerve agent toxicity, rats were fed four distinct diets *ad libitum* for 28 d prior to challenge with 110 μg/kg (1.0 LD_{50}, sc) soman. The four diets used were a standard rodent diet, a choline-enriched diet, a glucose-enriched diet, and a ketogenic diet. Body weight was recorded throughout the study. Toxic signs and survival were evaluated at key times for up to 72 h following soman exposure. Additionally, acquisition of discriminated shuttlebox avoidance performance was characterized beginning 24 h after soman challenge and across the next 8 d (six behavioral sessions). Prior to exposure, body weight was highest in the standard diet group and lowest in the ketogenic diet group. Upon exposure, differences in soman toxicity as a function of diet became apparent within the first hour, with mortality in the glucose-enriched diet group reaching 80% and exceeding all other groups (in which mortality ranged from 0 to 6%). At 72 h after exposure, mortality was 100% in the glucose-enriched diet group, and survival approximated 50% in the standard and choline-enriched diet groups, but equaled 87% in the ketogenic diet group. Body weight loss was significantly reduced in the ketogenic and choline-enriched diet groups, relative to the standard diet group. At 1 and 4 h after exposure, rats in the ketogenic diet group had significantly lower toxic sign scores than all other groups. The ketogenic diet group performed significantly better than the standard diet group on two measures of active avoidance performance. The exacerbated soman toxicity observed in the glucose-enriched diet group coupled with the attenuated soman toxicity observed in the ketogenic diet group implicates glucose availability in the toxic effects of soman. This increased glucose availability may enhance acetylcholine synthesis and/or utilization, thereby exacerbating peripheral and central soman toxicity.

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1. Introduction

Diet composition and nutritional status have been shown to influence the progression of diseases induced by toxic substances (Hennig et al., 2007; Shakman, 1974). Specific nutrient deficiencies can exacerbate the toxicity of many substances (Shakman, 1974). Furthermore, dietary supplementation with specific nutrients has been shown to mitigate the toxicity produced by a number of substances (Hennig et al., 2007; Shakman, 1974). Diet composition and nutrient levels can modify the physiological and neurobehavioral effects of pharmacological and toxicological compounds by altering neurological chemistry, metabolic processes, and pharmacokinetics (Fenech, 2005; Keenan et al., 1999; Nold et al., 2001). Diet content, as an independent variable in the effects of chemical warfare nerve agents (CWNA), has received little attention. However, the manipulation of diet content has received considerable attention in recent years, in both clinical and research applications, from those investigating seizure control in epilepsy disorders (Bough and Rho, 2007; Hartman et al., 2007).

Fasting has been used since antiquity as a treatment for the control of seizures (DeVivo et al., 1975; Hartman et al., 2007); however, the mechanism(s) through which fasting functions as an effective anticonvulsant is unknown. Fasting is known to induce metabolic changes, including decreased blood glucose and increased fatty acid metabolism, resulting in the production of ketone bodies (i.e., β-hydroxybutyrate, acetoacetate, and acetone) (DeVivo et al., 1975; Sokoloff, 1973) via the metabolic process known as ketosis. The ketogenic diet (KD) was introduced in the 1920s as an alternative to fasting (to produce the effects of fasting without starvation) for the control of seizures (DeVivo et al., 1975; Hartman et al., 2007). The KD, in its typical formulation, has a high percentage (by weight) of fats (≥70%), a moderate percentage of proteins (~20%), and a low percentage of carbohydrates (<10%). Similar to prolonged fasting, the KD has been shown to function as an effective treatment for epilepsy in clinical applications (for review see Bough and Rho, 2007). Furthermore, using a variety of

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animal models of epileptic disorders (6 Hz audiogenic seizures, pentylenetetrazole [PTZ], kainic acid [KA], pilocarpine, etc.), the KD has been shown to elevate seizure thresholds and/or have anticonvulsant properties (Stafstrom, 1999). The mechanism(s) by which the KD exerts its effects is unclear (Rho and Sankar, 2008). Under normal dietary conditions the brain derives most of its energy from the metabolism of glucose (glycolysis). However, during prolonged fasting or protracted consumption of a KD, blood glucose levels decrease due to reduced glycolysis, blood ketone body levels increase due to enhanced lipid metabolism, and the brain uses ketone bodies as an alternative energy source (Sokoloff, 1973). The beneficial effects of the KD on seizure control may be due to reduced glucose levels, increased ketone body production, increased free fatty acids, and/or altered neurotransmitter synthesis (Bough and Rho, 2007).

Support for a role of glucose in epileptic disorders comes from studies indicating that seizure control produced by the KD is rapidly reversed upon ingestion of carbohydrates (Bough and Rho, 2007). Similarly, other means of regulating glucose levels (e.g., fasting, caloric restriction) have been shown to have beneficial effects on the regulation of seizures (Greene et al., 2003; Maalouf et al., 2009). A few recent reports indicate that elevated sugar intake (glucose or high fructose corn syrup in drinking water) exacerbates the toxicity of parathion poisoning, an organophosphorus (OP) insecticide (Liu et al., 2005, 2007; Olivier et al., 2001). In Liu et al. (2005), adult rats that had consumed high fructose corn syrup in drinking water for 7 d prior to parathion exposure showed decreased weight gain, increased salivation, lacrimation, urination, and defecation (SLUD) signs and increased involuntary movements over a 7 d period following exposure. The study also demonstrated reduced toxicity in a group of rats maintained under caloric restriction compared to *ad libitum* fed rats and systematically replicated the increased toxicity of parathion in rats consuming sweetened water, providing further support for the role of glucose in OP toxicity.

As alluded to above, nutrient supplementation has been shown to either reverse or mitigate the effects of toxic insult (Hennig et al., 2007; Shakman, 1974). Administration of choline or choline analogues has been shown to influence the action of both anticholinesterases (Patterson et al., 1989; Stovner, 1956) and anticholinergics (Wecker et al., 1978). Furthermore, there appears to be a causal relationship between choline availability during development and cognitive function in adulthood (McCann et al., 2006). Dietary choline supplementation has been shown to reduce spatial memory impairments and hippocampal cell loss induced by KA seizures (Holmes et al., 2002) as well as to improve the behavioral, histological, and neurochemical outcomes associated with traumatic brain injury (Guseva et al., 2008).

The doctrinal treatment of CWNA poisoning for U.S. military personnel consists of the rapid administration of an anticholinergic (e.g., atropine sulfate) in conjunction with an oxime reactivator (e.g., pralidoxime, 2-PAM). Additionally, if the signs and symptoms warrant, a benzodiazepine (e.g., diazepam) is administered to control seizures induced by overstimulation of cholinergic synapses (Sidell et al., 2008). Ideally, these post-exposure treatments would be supplemented by a prophylactic regimen consisting of a reversible acetylcholinesterase inhibitor (e.g., pyridostigmine) that would sequester (reversibly inhibit) approximately 30% of the available acetylcholinesterase (Sidell et al., 2008) prior to nerve agent exposure. The current focus of research groups worldwide appears to be investigating compounds from these three pharmacological classes (i.e., anticholinergics, oxime reactivators, and benzodiazepines) that will have greater efficacy in preventing or reducing the sequelae associated with CWNA poisoning (Bajgar et al., 2009; McDonough et al., 2009; Shih et al., 2007; Wetherell et al., 2007). However, a few novel approaches to the management of CWNA poisoning include the use of exogenously administered enzymes that function as either stoichiometric or catalytic bioscavengers (Lenz et al., 2007; Saxena et al., 2006). Others are investigating the use of compounds that antagonize both cholinergic and glutamatergic synapses to reduce seizure activity and the resultant neuropathology associated with prolonged seizures (Myhrer et al., 2008, 2010). Others in the field are investigating the use of centrally active acetylcholinesterase inhibitors (i.e., galantamine, huperizine, and physostigmine) as alternatives to pre-treatment with pyridostigmine (Aracava et al., 2009; Haigh et al., 2008; Lallement et al., 2002; Wetherell et al., 2007). There have also been considerable efforts to develop a broad-spectrum oxime for the effective treatment of poisoning by CWNAs of different structural and chemical compositions (Kassa et al., 2010; Kuca et al., 2010).

There have been few studies to examine the influence of dietary variables on CWNA toxicity. Two relevant studies have examined the impact of short-term fasting (~18–24 h) (Fletcher et al., 1988b; Myers et al., 2005) and both found exacerbated toxicity of CWNA following fasting. However, as revealed in the epilepsy literature, prolonged ($\geq$48 h), but not short-term ($\leq$24 h), fasting has an anticonvulsant effect. In the present study, we examined the effects of the KD, a glucose-enriched diet, and a choline-supplemented diet on CWNA toxicity by evaluating toxic signs, body weight changes, and two-way active avoidance responding following an acute CWNA exposure. It was expected that the KD would produce metabolic effects similar to prolonged fasting (without starvation and weight loss) and have a protective effect against CWNA toxicity. Similarly, we expected the increased availability of monosaccharides in the glucose-enriched diet to exacerbate CWNA toxicity. Finally, given the encouraging results from choline supplementation studies, we predicted that choline supplementation would have beneficial effects on recovery of function following CWNA insult.

2. Method

2.1. Subjects

Sixty (60) male Sprague–Dawley rats (Crl:CD(SD)) were obtained from Charles River Laboratories (Wilmington, MA, USA). Rats weighed between 175 and 200 g at the time of arrival and were acclimated to our facilities and observed for evidence of disease for 5 d prior to initiating the study. Rats were implanted subcutaneously (sc) with sterile transponders (IPTT-300; BioMedic Data Systems Inc., Seaford, DE, USA) for animal identification and fed a standard rodent diet *ad libitum* until the dietary variable was implemented. Throughout the study, the rats were pair-housed in polycarbonate cages with *ad libitum* water in fully AAALAC accredited facilities under a 12-h light/dark cycle (lights on at 0600) with temperature ($21 \pm 2\,^{\circ}C$) and relative humidity ($50 \pm 10\%$) controlled.

2.2. Apparatus

Active avoidance training was conducted in eight commercially available chambers (Gemini System, San Diego Instruments Inc., San Diego, CA, USA). Each avoidance chamber had exterior dimensions of 66 cm (W) $\times$ 33 cm (D) $\times$ 44 cm (H) and was composed of two compartments each measuring 24 cm (W) $\times$ 20 cm (D) $\times$ 20 cm (H). Each compartment was equipped with a cue light centered on the distal wall and 12.5 cm above the grid floor, a house light centered over the compartment, and a speaker located in the front distal corner of the ceiling. Each compartment was equipped with eight infrared emitter-receiver

pairs arranged in a yoked manner for detecting the compartment in which the rat was located.

2.3. Procedure

2.3.1. Diet implementation and soman exposure

Rats were equally and randomly assigned to one of four diet groups: *standard, glucose, choline, or ketogenic*. The rats were maintained on their respective diet for 28 d prior to challenge with soman (1.0 LD_{50}, sc). Soman (GD; pinacolyl methyl phosphonofluoridate; >98% purity) was obtained from the Edgewood Chemical Biological Center (Aberdeen Proving Ground, MD, USA), diluted in ice-cold sterile saline, and stored at −80 °C until the day of use. Body weights were collected each weekday. On the 28th d of diet consumption, each rat was administered a single sc injection of soman (220 μg/ml) in a volume of 0.5 ml/kg.

2.3.2. Toxic signs assessment

Toxic signs were evaluated at 1, 4, 24, 48, and 72 h after exposure. Toxic signs assessments were made by making visual observations for motor dysfunction, general coordination, excessive mastication, salivation, lacrimation, and piloerection. The presence or absence of salivation, mastication, piloerection, and lacrimation were scored separately as "1" if present and "0" if absent. Additionally, a four-point scale (0–3) was used to measure movements associated with cholinergic overstimulation (normal, fasciculations, tremor, and convulsions) and ambulatory/righting ability (normal, impaired movement, and prostrated/immobile). Thus, the maximum toxic signs score a rat could receive was 10.

2.3.3. Active avoidance testing

At 24 h after exposure, active avoidance training began. A discriminated shuttlebox avoidance test was used. Each avoidance session began with a 5-min acclimation period during which all chamber illumination was off. Following the acclimation period, a maximum of 50 discrete trials were presented. Each trial began following the determination of the rat's location. Thereafter the house and cue lights in that compartment were illuminated and served as the warning stimulus (WS). If the rat ambulated completely into the darkened compartment within 5 s following the onset of the WS, the trial was terminated and scored as an "avoidance response." If, however, the rat failed to move into the darkened compartment within 5 s of the onset of the WS, the grid floor was electrified with a scrambled 1.0-mA shock with a frequency of 1.0 Hz (0.5 s shock on, 0.5 s off) for a maximum of 15 s. This shock served as the aversive stimulus (AS). If the rat moved into the darkened compartment following AS onset, the trial was scored as an "escape response." Finally, if the rat failed to cross into the darkened chamber within 15 s of AS, the trial was terminated and scored as a "no response." If the rat had 10 consecutive "no response" trials, the session was terminated. Trials were separated by an intertrial interval (20 ± 5 s). On all trials, the response latency determined the duration of the trial and was recorded. Due to excessive "gate behavior" (rats straddling compartments at the onset of the WS), the AS was applied to both compartments from the third day of avoidance training to the completion of the experiment. Experimental events and data collection were accomplished using in-house software written in Visual Basic® 6.0 (Microsoft Corporation, Redmond, WA, USA) with a resolution of 0.01 s.

2.3.4. Diets

The standard diet was Harlan Teklad Certified Rodent Diet 8728C. The glucose-enriched diet was Harlan Teklad Custom Research Diet TD.05256. This was a modified version of Teklad TD.89247 with the fructose isocalorically replaced with dextrose (glucose). The KD was Bio-Serv #F3666. The choline-enriched diet was Harlan Teklad Certified Rodent Diet 8728 C with 17 g/kg choline chloride (Teklad TD.09416). The choline diet contained about seven times as much choline as the standard diet (2.53 g/kg). Table 1 shows select nutrient profiles for the four diets used. Due to slowed growth rates in the KD group, 250 g powdered soy protein (The Vitamin Shoppe®, Item #VS-1621 1464205, North Bergen, NJ, USA; 86.67% protein, 2% fat, 0% carbohydrates) was thoroughly mixed into each kg of the KD paste on days 10–18. Beginning on day 19, 240 g powdered casein protein (Bio-Serv, Product #1100, Frenchtown, NJ, USA; 89.4% protein, 1% fat, and 0.1% carbohydrates) was added to each kg of the KD (in place of the soy protein; consistent with Ziegler et al., 2002). The addition of either protein source was successful in maintaining an increased growth rate while preserving the low proportion of carbohydrates required of the KD.

2.4. Statistical analysis

Survival analyses were conducted using S-PLUS® 7.0 software (Insightful Corporation, Seattle, WA, USA) using the *survfit* function, and differences between groups at each time point were evaluated using the *survdiff* function. Adjustments to the critical p values for multiple comparisons were made using the Sidak correction procedure. This approach fits a Kaplan–Meier survival curve and compares groups based on the Fleming–Harrington G^{ρ} family of tests (*S-PLUS 7 Guide to Statistics, Volume 2*, Insightful Corporation, Seattle, WA, USA, 2005). Growth rate (final 7 d of baseline body weights) differences were analyzed by fitting a linear mixed effects model (SPSS® MIXED©) with diet, day and their interaction as fixed effects, day as the repeated effect with compound symmetry covariance structure, and subject as the random effect. Post-exposure body weight changes were evaluated using a two-way repeated-measures analysis of variance (ANOVA) with diet as the between-subjects factor and post-exposure day as the repeated measure using SPSS® 12.0 (SPSS Inc., Chicago, IL, USA). Toxic signs data were analyzed via a linear mixed effects model (SPSS® MIXED©) with diet as the between-subjects factor and day as the repeated measure (subject was a random factor and the repeated-measures covariance structure was compound symmetry). Active avoidance data (average time spent in the AS per trial and the proportion of avoidance responses) were analyzed by linear mixed effects models (SPSS® MIXED©) with diet as the between-subjects factor and post-exposure session as the repeated factor (subject was a random factor and the covariance structure of the repeated measures was compound symmetry).

Table 1
Selected nutrient profiles for test diets.

	Harlan Teklad 8728C (standard diet)	Harlan Teklad TD.09416 (choline diet)	Harlan Teklad TD.05256 (glucose diet)	Bio-Serv F3666 + 1100 (ketogenic diet)
Protein	24.5 (32.4)	24.1 (32.5)	18.3 (20.2)	27.2 (14.3)
Carbohydrates	40.9 (54.2)	40.2 (54.2)	60.4 (66.8)	0.7 (0.4)
Fat	4.5 (13.4)	4.4 (13.3)	5.2 (12.9)	72.1 (85.3)

Values represent percentages by weight with corresponding numbers in parentheses showing percent kcal.

3. Results

3.1. Growth rates

The effect of the four diets on growth rate prior to agent challenge is depicted in Fig. 1. Initially, body weights were comparable across the four diet groups, but differences began to become apparent as early as day 3. Specifically, the mean body weight was lower in the KD group than in any other group. The addition of soy protein to the KD increased growth to a rate that was comparable to the three other diets but insufficient to overcome the early differences in body weight. The addition of casein produced no discernible change in rate of growth. By the final day prior to soman exposure, differences in mean body weight were apparent between groups. Specifically, the standard diet produced the highest mean body weight, followed closely by the glucose and choline diets. The KD produced the lowest mean body weight. The analysis of the final seven body weights obtained during baseline revealed significant main effects of diet [$F(3, 56) = 22.11$, $p < .001$] and day [$F(6, 336) = 542.66$, $p < .001$]. There was no significant interaction between diet and day [$F(18, 336) = 1.04$, $p > .4$]. Rats fed the standard diet weighed significantly more than those fed either the choline-enriched or the ketogenic diet. Rats fed the KD weighed significantly less than those fed all other diets. The main effect of day confirmed the visual observation that body weights increased daily for all groups throughout the final seven measurements.

3.2. Survival

One hour following challenge with 1.0 LD_{50} soman, 80% (12/15) of the rats fed the glucose diet had died, and one rat each from the standard and choline diet groups had died. By 24 h following challenge, the percentage of rats surviving in each group was 53.3% (8/15) in standard, 6.7% (1/15) in glucose, 60% (9/15) in choline, and 93.3% (14/15) in ketogenic. By 48 h following soman challenge, survival in each diet group was 40% (6/15) in standard, 0% (0/15) in glucose, 53.3% (8/15) in choline, and 86.7% (13/15) in ketogenic. The percentage of rats surviving in each group at 72 h was the same as that at 48 h. Comparison of the survival rates at 72 h after soman challenge revealed that the percentage of rats surviving from the glucose group was significantly less than that of all other groups.

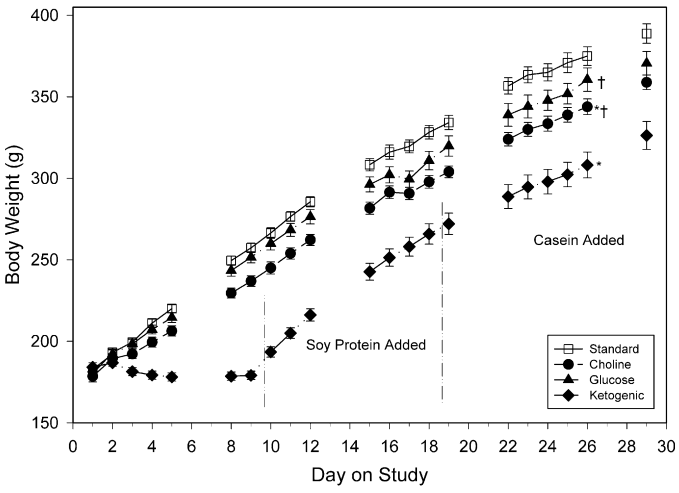

Fig. 1. Effects of specialized diets on growth rate of rats. Analysis of the final seven measurements revealed that rats fed the standard diet were significantly heavier than those fed either the choline-enriched or the ketogenic diet. Furthermore, rats fed the KD weighed significantly less than those fed all other diets. Data are the mean of 15 rats/diet group. Curves labeled with "*" are significantly different from the standard diet group. Curves labeled with "†" are significantly different from the KD group.

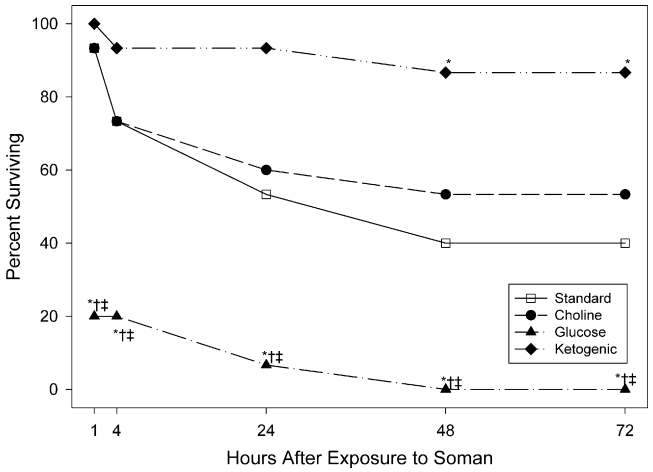

Fig. 2. Survival curves for each of the four different diet groups throughout the first 72 h following challenge with 1.0 LD_{50} soman. At 1 h after soman, only 20% of the rats fed the glucose-enriched diet had survived, and by 48 h there were no surviving glucose diet rats. In contrast, at 72 h, 86.7% of the KD rats had survived. Points labeled with "*" indicate a significant difference ($p < .05$) from the standard diet group. Points labeled with "†" and "‡" indicate significant differences from choline and ketogenic diet groups, respectively ($n = 15$/diet).

Further, the percentage surviving in the standard group was significantly less that than in the KD group. There were no significant differences between the percentages of rats surviving from the choline group and either the standard or the ketogenic group. Fig. 2 shows the survival curves for all four diet groups up to 72 h after soman challenge.

3.3. Percent control body weight

Percent control body weights were determined by dividing an animal's weight on a given post-exposure day by that animal's weight just prior to exposure and then multiplying the dividend by 100. The glucose diet group had no survivors and was excluded from this analysis. A two-way repeated measures ANOVA was conducted and revealed a significant main effect of diet [$F(2, 60) = 24.24$, $p < .001$], a significant main effect of day [$F(6, 149) = 20.95$, $p < .001$], and a significant interaction between day and diet [$F(12, 149) = 9.49$, $p < .001$]. Dunnetts' post hoc comparison revealed that the standard diet group had significantly lower percent control body weights than either the choline or the ketogenic group. Across groups, body weights had decreased by approximately 10% at 24 h after soman challenge. Across days, rats in the KD group had significantly higher percent control body weights than did rats in either the choline or the standard diet group. Furthermore, beginning on the third and continuing through the seventh post-exposure day, the choline diet group had significantly higher percent control body weights than did the standard diet group. Fig. 3 shows these body weight changes for 7 d after soman challenge.

3.4. Toxic signs

Signs of soman poisoning were evaluated for each rat at 1, 4, 24, 48, and 72 h after soman challenge. The main effect of diet was significant [$F(3, 56) = 7.68$, $p < .001$], and post hoc comparisons revealed that rats in the glucose group had significantly higher toxic sign scores than did the rats in all other groups. Further, rats in the KD group had significantly lower toxic sign scores than rats in all other groups. There was also a significant main effect of hour [$F(4, 111) = 35.27$, $p < .001$]. Toxic sign scores at the 1- and 4-h time points were significantly greater than those at the later hours. There was a significant interaction between diet and hour [$F(10,$

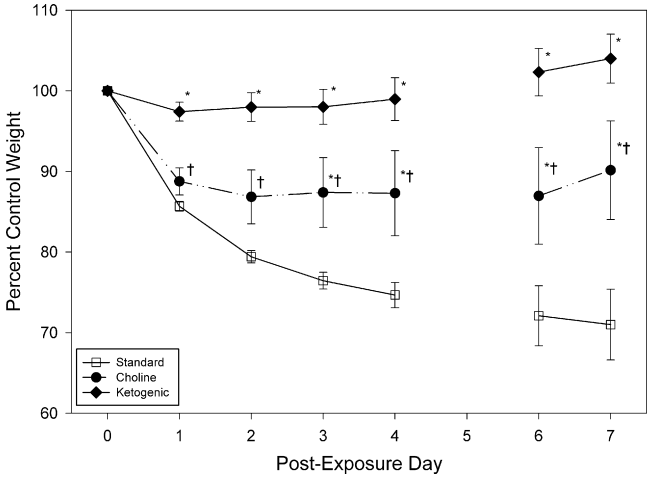

Fig. 3. Percent control body weight ± standard error of the mean (SEM) as a function of day after soman challenge (n = 6–15/diet group depending upon survival; see Fig. 2). Rats were challenged with 1.0 LD_{50} soman on day 0. Rats fed the KD and those fed the choline diet had significantly less weight loss following soman challenge than rats fed the standard diet. Points labeled with "*" and "†" were significantly different ($p < .05$) from the standard and KD groups, respectively.

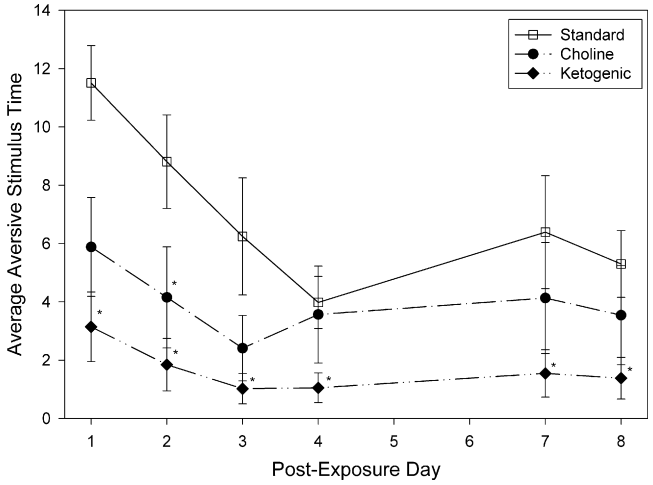

Fig. 5. Active avoidance acquisition following soman challenge. Average aversive stimulus (AS) time per trial as a function of post-exposure day. Overall, rats fed the KD spent significantly less time in the AS than did rats fed the standard diet. Points labeled with "*" were significantly different from those of the standard diet group (n = 6–15/diet group).

111) = 6.89, $p < .001$]. Tests of simple main effects revealed that at the 1- and 4-h time points, rats in the KD group had significantly lower toxic sign scores than did rats in all other groups. Furthermore, for all groups except the KD group, toxic sign scores at 1 and 4 h were significantly higher than those at 24, 48, and 72 h. (Due to mortality, there were no toxic sign scores available for rats in the glucose group past the 24-h time point.) Fig. 4 shows the toxic signs score for each diet group at 4 h after soman challenge.

3.5. Active avoidance performance

Two measures were used to evaluate shuttlebox avoidance performance for each session: the average time spent in the AS per trial (AST) and the proportion of avoidance responses emitted. Due to violating the assumption of homogeneity of variances, the average AS time data were log10 transformed prior to the ANOVA. There was a significant effect of diet [$F(2, 26) = 8.41$, $p < .001$] on the average AST. This measure indexes the rat's proficiency in avoiding and escaping the AS. Post hoc comparisons indicated that rats in the KD group spent significantly less time in the AS than did rats in the standard diet group. There was also a significant effect of post-exposure day [$F(5, 119) = 16.86$, $p < .001$]. AST was significantly higher on post-exposure day 1 than in all later sessions. AST on post-exposure day 2 was also significantly higher than that observed on post-exposure days 3 and 4. There was no significant interaction between diet and post-exposure day [$F(10, 119) = 0.84$, $p > .5$]. Fig. 5 shows mean AST for each of three diet groups across the eight post-exposure days (six sessions).

Due to violating the assumption of homogeneity of variances, the proportion of avoidance responses were arcsine transformed prior to the ANOVA. The ANOVA revealed a significant main effect of diet [$F(2, 27) = 5.12$, $p = .013$], a significant main effect of post-exposure day [$F(5, 120) = 5.53$, $p < .001$], and no significant interaction between diet and post-exposure day [$F(10, 120) = 1.42$, $p > .15$]. Post hoc analyses revealed that rats in the KD group made significantly more avoidance responses than did rats in the standard diet group. The main effect of post-exposure day indicated that the proportion of avoidance responses made during the session on the first post-exposure day were significantly less than those made during sessions on post-exposure days 2–3 and 7–8. Fig. 6 shows the

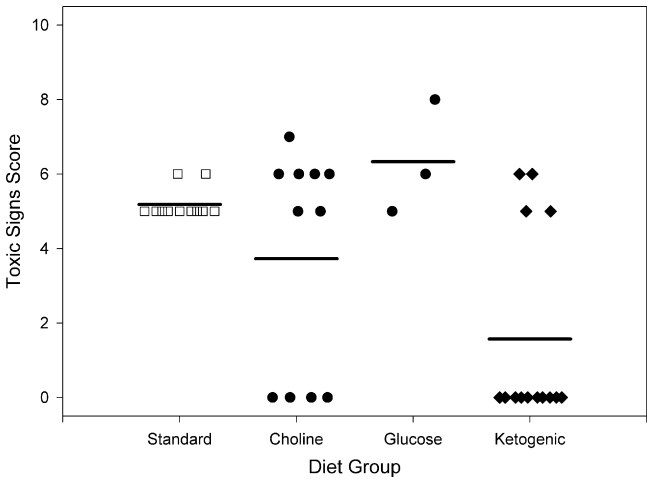

Fig. 4. Signs of soman toxicity 4 h after soman challenge. Each point represents an individual rat in that respective diet group. Solid horizontal lines above each group label represent the group mean toxic signs score. The toxic signs scores of the KD group were significantly less than those of all other groups.

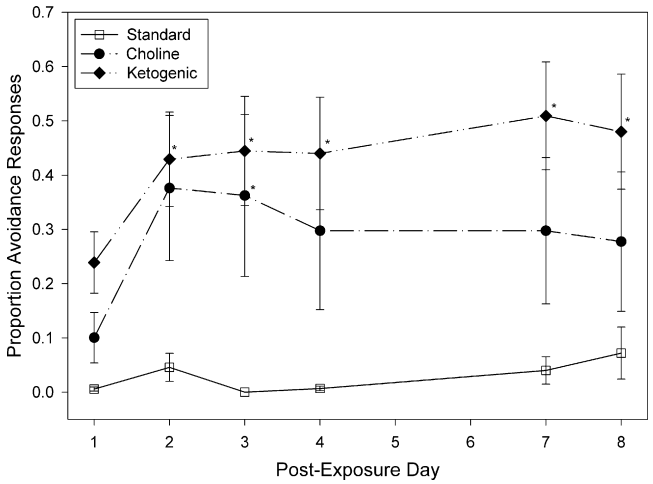

Fig. 6. Proportion of trials per session with an avoidance response (±SEM) as a function of day after soman challenge. Rats fed the KD made significantly more avoidance responses than did rats fed the standard diet. Points labeled "*" were significantly different from the standard diet group. (n = 6–14/diet group).

proportion of avoidance responses for each of three diet groups across eight post-exposure days.

4. Discussion

The present data indicate that rats maintained on a diet high in glucose show increased sensitivity to the toxic and lethal effects of soman when compared to rats fed other diets. The glucose-enriched diet exacerbated toxic signs and made a 1.0 LD_{50} dose of soman functionally equivalent to a 1.0 LD_{100} dose. In stark contrast, rats maintained on the KD had higher 72-h survival rates than all other diet groups. Additionally, the KD appeared to confer protection against the toxic effects of soman because rats in the KD group had significantly reduced toxic signs and less weight loss than rats in either the choline or the standard diet group. Finally, the protective effects of KD were further demonstrated during the acquisition of a two-way shuttlebox avoidance task designed to assess neurobehavioral function. Rats fed the KD emitted significantly more avoidance responses and spent significantly less time in the AS than rats fed the standard diet.

The exacerbated toxicity observed in rats fed a glucose-enriched diet in the present study following challenge with a 1.0 LD_{50} dose of soman is in agreement with results from previous studies (Liu et al., 2005, 2007; Olivier et al., 2001) that demonstrated exacerbated toxicity of the OP pesticide parathion in rats given drinking water with high fructose corn syrup or glucose added. Furthermore, deldrin toxicity has been shown to increase following glucose administration in adult rats (Fox and Virgo, 1986). The specific mechanism(s) by which glucose or fructose exacerbates the toxicity of OP compounds is unknown. However, glucose may facilitate the synthesis and/or release of acetylcholine (Kopf and Baratti, 1996; Ragozzino et al., 1996), thereby increasing the toxicity of acetylcholinesterase (AChE) inhibitors such as soman and parathion. Indeed, glucose administration has been shown to potentiate the effects of the AChE inhibitor physostigmine and attenuate the effects of the anticholinergic scopolamine (Stone et al., 1988a,b). Alternatively, human diabetics (Aoyagi et al., 1985) and experimentally induced diabetic rats have been shown to have decreased AChE activity (Agarwal et al., 1985; Dash et al., 1991) and diazinon produced greater toxicity in streptozotocin-induced diabetic rats than in normal rats (Ueyama et al., 2007). Furthermore, serum paraoxonase (PON) activity is decreased in streptozotocin-induced diabetic rats (Patel et al., 1990) and in diabetic humans (Mackness et al., 2002). Moreover, *in vitro* carboxylesterase is inactivated in the presence of a variety of sugars, this is due to glycation (Yan and Harding, 1999, 2003, 2005). The exacerbated soman toxicity in the glucose-enriched diet group in the present study could be due to the inactivation of esterases (AChE, PON, CaE, or butyrylcholinesterase) by glycation and/or glyoxidation. The interaction between glucose and the cholinergic system appears to be bidirectional. OP compounds are known to induce hyperglycemia, but this effect has generally been ascribed to a stress response (Fletcher et al., 1988a,b; Kant et al., 1988; Peoples et al., 1988; Seifert, 2001). However, direct injection of neostigmine into the hypothalamus elevates blood glucose and epinephrine levels and this effect is likely due to the inhibition of AChE in the hypothalamus (Honmura et al., 1992).

Rats fed a choline-enriched diet were predicted to have enhanced recovery of function following soman challenge relative to those fed a standard diet. Choline supplementation has been shown to enhance behavioral recovery following either traumatic brain injury (Guseva et al., 2008) or seizures induced by pilocarpine (Yang et al., 2000) and KA (Holmes et al., 2002) without differences in seizure severity or lethality. Furthermore, choline supplementation has been reported to decrease the incidence of seizures and the lethality of nicotine, paraoxon, and PTZ (Wecker et al., 1982). The mechanism responsible for these effects is unclear; however, it is recognized that choline is a selective agonist at the $\alpha 7$ nicotinic cholinergic receptor and chronic supplementation can result in up-regulation of these receptors (Guseva et al., 2008). Choline supplementation also enhances acetylcholine synthesis selectively during increased neuronal demand (Koppen et al., 1997; Wecker, 1986) but not under basal conditions. In the present investigation, the choline-enriched diet did appear to confer a modest benefit following soman challenge. Specifically, body weight was significantly greater than that in the standard group on post-exposure days 3, 4, 6, and 7. Additionally, avoidance performance was somewhat enhanced relative to the standard diet group in the early days after soman exposure (AS time was significantly lower on post-exposure day 2, and avoidance responding was significantly higher on post-exposure day 3). Taken together, these findings do suggest that added dietary choline may confer a modicum of benefit, although the mechanism is unclear and the results require replication.

Rats maintained on a KD for four weeks prior to soman challenge exhibited increased survival, decreased toxic signs, decreased weight loss, and increased two-way shuttlebox avoidance performance compared to rats fed the standard diet. This study is the first to demonstrate that the toxicity of CWNA may be attenuated by a high-fat diet. Currently, the mechanism responsible for this effect is not known. The KD, however, has been shown to induce alterations in gene expression associated with metabolic processes (Bough et al., 2006) in addition to possessing both anticonvulsant and neuroprotectant properties (Bough and Rho, 2007; Greene et al., 2003; Hartman et al., 2007; Maalouf et al., 2009; Schwartzkroin, 1999). Recently, it was shown that feeding a KD to adult rats dosed with parathion during the neonatal period ameliorated the effects of exposure on certain parameters of ACh function (Slotkin et al., 2009). The anticonvulsant and neuroprotectant properties of the KD have been proposed to be due to its antioxidant properties (Bough and Rho, 2007; Maalouf et al., 2009), the direct anticonvulsant actions of ketone bodies (Gasior et al., 2007; Maalouf et al., 2009; Rho et al., 2002), and altered neurotransmitter (GABA) synthesis (Bough and Rho, 2007; Hartman et al., 2007; Maalouf et al., 2009). Another potential mechanism for the protective effects of the KD is that it reduces glucose availability and utilization, thereby conferring a protective benefit (DeVivo et al., 1975; Greene et al., 2001, 2003; Mantis et al., 2004; Schwechter et al., 2003). The fat content of the KD in the current investigation consisted of approximately 38% oleic acid, which has been shown to protect paraoxonase 1 (PON1) from oxidative inactivation (Costa et al., 2005). In a separate investigation, the addition of triolein to the diet of rats increased serum PON1 activity by over 40% compared to a control diet (Kudchodkar et al., 2000). Furthermore, it has been shown that in rats, both the amount and type of fat in the diet has an influence on both serum carboxylesterase (ES-1) and butyrylcholinesterase activity (Van Lith et al., 1989, 1990, 1991). All three of these enzymes are capable of detoxifying nerve agents (Costa et al., 2005; Lenz et al., 2007; Maxwell et al., 1987) and the KD's protective benefits may due to increased activity of any one of these enzymes.

The present investigation revealed that consumption of a glucose-rich diet exacerbated the toxic and lethal effects of soman. In contrast, consumption of the KD diminished the toxic and lethal effects of soman. The mechanisms responsible for the opposing effects of the different diets remain elusive. Further research is needed to determine the mechanism(s) responsible for the protective effects of the KD. Such studies could be beneficial for understanding the influence of metabolic process on nerve agent toxicity and aid in the development of new pharmacological or dietary countermeasures against nerve agent intoxication.

Conflict of interest

None.

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DISCLAIMER

The opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the U.S. Army or the Department of Defense.

The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense and all procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996), and the Animal Welfare Act of 1966 (P.L. 89-544), as amended.

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